Original article

Title: Phenotypic integration and life history strategies among populations of *Pinus halepensis*: an insight through Structural Equation Modelling

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ABSTRACT

- *Background and Aims*

Understanding inter-population variation in the allocation of resources to specific anatomical compartments and physiological processes is crucial to disentangle adaptive patterns in forest species. This work aims to interpret phenotypic integration and trade-offs among functional traits as determinants of life history strategies in populations of a circum-Mediterranean pine that dwells in environments where water and other resources are in limited supply.

- *Methods*

Adult individuals of 51 populations of *Pinus halepensis* grown in a common garden were characterized for 11 phenotypic traits, including direct and indirect measures of water uptake at different depths, leaf area, stomatal conductance, chlorophyll content, non-structural carbohydrates, stem diameter and tree height, age at first reproduction and cone production. The population differentiation in these traits was tested through ANOVA. The resulting populations' means were carried forward to a Structural Equation Model testing for phenotypic integration between six latent variables (summer water uptake depth, summer transpiration, spring photosynthetic capacity, growth, reserve accumulation and reproduction).

- *Key Results*

Water uptake depth and transpiration co-varied negatively among populations, as the likely result of a common selective pressure for drought resistance, while spring photosynthetic capacity was lower in populations originating from dry areas. Transpiration positively influenced growth, while growth was negatively related to reproduction and reserves among populations. Water uptake depth negatively influenced reproduction.

- *Conclusions*

  The observed patterns indicate a differentiation in lifecycle features between fast-growing and slow-growing populations, with the latter investing significantly more in reproduction and reserves. We speculate that such contrasting strategies result from different arrays of life history traits underlying the very different ecological conditions that the Aleppo pine must face across its distribution range. These comprise, principally, drought as main stressor and fire as main ecological disturbance of the Mediterranean basin.

Keywords: evolutionary diversification; fitness; integrated phenotype; life history; *Pinus halepensis*; trade-offs; Structural Equation Modelling

INTRODUCTION

Disentangling the extent and nature of morpho-physiological adaptations is a current issue for understanding ecosystem functioning and forest dynamics in the context of global change (Guittar *et al.*, 2016; Kunstler *et al.*, 2016). Although common-garden tests have been used for a long time to characterize the genetic differentiation in functional traits for many forest species, the study of phenotypic integration for understanding adaptation syndromes has received little attention thus far (Savolainen *et al.*, 2007; Bussotti *et al.*, 2015). At the intra-specific level, phenotypic integration commonly defines the disposition of several traits to evolve jointly during the divergence of populations (i.e., evolutionary integration; Armbruster *et al.*, 2014). Indeed, the allocation of resources to a particular plant compartment or physiological process impacts on the overall carbon economy of a tree, potentially involving multiple trade-offs (Milla *et al.*, 2011). Understanding patterns of phenotypic integration is thus crucial to disentangle contrasting adaptive strategies among populations (Murren 2002).

The Aleppo pine (*Pinus halepensis* Mill.) is a widespread circum-Mediterranean gymnosperm predominantly distributed in the central-western part of the Mediterranean basin (Fady *et al.*, 2003). It is a drought-avoidant species that can be found under very contrasting ecological conditions which have shaped its current patterns of genetic variation (Serra-Varela *et al.*, 2017). Based on common-garden tests, genetic differentiation among populations has been described in life history traits such as total growth (Schiller and Atzmon, 2009; Voltas *et al.*, 2018) and reproductive allocation (Santos-del-Blanco *et al.*, 2013), and also in functional traits related to drought resistance including hydraulic conductivity (Tognetti *et al.*, 1997), needle physiology (Klein *et al.*, 2013) and water uptake patterns (Voltas *et al.*, 2015). Together with drought stress, fire has been identified as major evolutionary force in Aleppo pine. *P. halepensis* has been classified as 'fire embracer', i.e., with population resilience

based on efficient post-fire recruitment after crown fires (Pausas, 2015). Differences in the frequency and intensity of forest fires across the range of *P. halepensis* have been associated with population differentiation in key traits like cone serotiny, aerial cone bank and bark thickness, and also with differences in the allocation of resources to growth or reproduction (Santos-del-Blanco *et al.*, 2013; Hernández-Serrano *et al.*, 2014; Martín-Sanz *et al.*, 2016 and submitted).

Despite the abundant information about genetic differentiation in functional and life history traits among populations of *P. halepensis*, little attention has been devoted to disentangle patterns of phenotypic integration in this species. Here, we use Structural Equation Modeling (SEM) to test the hypothesis of phenotypic integration among several functional and life history traits using a representative set of populations of the species (Grace *et al.*, 2010; Fan *et al.*, 2016). Our conceptual model is reported in Fig.1 and is grounded on current scientific evidence. Broadly speaking, the tree carbon economy depends on the balance between source-related processes (i.e., those influencing the amount of carbon that is available for the plant) and carbon sinks (i.e., determining the use of carbon resources) (Lacointe 2000).

In a drought-avoidant conifer such as *P. halepensis*, a key source-related trait varying among populations is photosynthetic rate, which is mainly determined by stomatal regulation rather than by biochemical limitations to photosynthesis (Santini *et al.*, 2019). Some populations exhibit reduced leaf area and tight stomatal regulation as adaptations to control transpiration and water losses, hence limiting the amount of carbon fixed through photosynthesis (Voltas *et al.*, 2008; Santini *et al.*, 2019). On the other hand, during periods of high water availability (i.e., spring) variation in photosynthetic capacity related to the needle biochemical composition may also play an important role in the carbon economy of Aleppo pine (Klein *et al.*, 2013). Populations with enhanced photosynthetic capacity may increase

their carbon fixation at the beginning of the growing season, when water availability is non-limiting to photosynthesis. Another source-related variable in Mediterranean species is water uptake depth. Although a deeper water uptake may imply a greater investment in rooting depth, which is a carbon-consuming (i.e., sink) process, it is also a key determinant of water supply under drought (Eggemeyer *et al.*, 2008; Rossatto *et al.*, 2012), which positively influences tree growth (e.g., through increased meristematic activity; Körner, 2003). Genetic variation in source-related traits such as transpiration, photosynthetic efficiency and water uptake depth has been described among populations of *P. halepensis* as adaptive mechanisms to cope with drought stress (Voltas et al. 2008; 2015; Santini et al. 2019). However, the genetic associations among these traits remain unexplored. As functional traits likely shaped by the same selective process (i.e., drought stress), a high phenotypic integration among these variables may have emerged linked to population differentiation (Armbruster *et al.*, 2014).

Phenotypic variation in source-related traits is associated with resource availability influencing carbon sinks. Typical carbon sinks of forest trees include growth, reproduction and storage, which are determinant traits of individual fitness largely influenced by the total amount of available resources (Lacointe 2000; Ryan et al. 2018). For instance, the effect of investment in root biomass or total water use on growth has been reported for several forest species (Oleksyn *et al.*, 1999; Körner, 2003; Voltas *et al.*, 2008; Klein et al. 2013; Santini *et al.*, 2019). While several studies have investigated the association between pairs of source-sink processes in forest species, the influence of each source-related variable on the different carbon sinks is not easily predictable in the context of a multi-trait analysis (Granier and Vile, 2014). A further complication arises from co-variation among plant sinks (e.g. Santos-del-Blanco *et al.*, 2012; Wiley and Helliker, 2012). In particular, growth, reproduction and storage must compete for limited carbon resources, resulting in trade-offs between resource-consuming processes. The growth *versus* reproduction trade-off is well known in forest trees,

and reflects divergent adaptive strategies among populations (Obeso, 2002; Climent *et al.*, 2008; Santos-del-Blanco *et al.*, 2014). Individuals growing in unstable environments tend to invest more resources in reaching a faster sexual maturity at the expense of slower growth, and vice versa (Niklas and Enquist, 2002; Santos-del-Blanco *et al.*, 2013). Indeed, previous studies described fast-growing populations of Aleppo pine characterized by a delayed sexual maturity, in contrast to slow-growing populations which invest more resources in reproduction (Climent et al. 2008). Reserve accumulation may also occur at the expense of growth, perhaps reflecting a higher resilience, as observed in populations exposed to unstable growing conditions (Wiley and Helliker, 2012; Granda and Camarero, 2017).

In this study, adult individuals of 51 population of *P. halepensis* growing in a common garden were characterized for 11 different morpho-physiological traits, which were used to gain information on three source-related variables (summer water uptake depth, summer transpiration and spring photosynthetic efficiency) and three carbon sinks (growth, reproduction and reserves) (Fig. 1). As derived by the conceptual model described above, we aim at testing the multivariate hypothesis of a direct influence of source-related processes on carbon sinks (SEM latent variables), assessing phenotypic integration (i.e., co-variation) within each group of latent variables. Specifically, we hypothesize 1) the existence of causal effects of source-related variables on carbon sinks, as suggested in the literature (e.g. Oleksyn *et al.*, 1999; Körner, 2003; Voltas *et al.*, 2008; Klein et al. 2013; Santini *et al.*, 2019), 2) the presence of patterns of free covariance among source-related variables, presumably as the result of phenotypic integration influenced by common selective pressures related to water scarcity, and 3) the relevance of sizeable free covariance among carbon sinks pointing to negative associations (i.e., trade-offs) between life history traits, as the result of different patterns of resource allocation among populations (Climent *et al.*, 2008). The objective of our work is to test these hypotheses through SEM using a number of phenotypic traits that

underlie the latent variables considered in the model. Aiming at producing an effective description of phenotypic integration, we also test simplified models by targeting only relevant associations among latent variables.

MATERIAL AND METHODS

Study site and plant material

The study was based on a multi-trait characterization of adult individuals of *P. halepensis* planted in a common-garden trial located in Altura (39° 49′ 29″ N, 00° 34′ 22″ W, 640 m a.s.l.; Castellón province, eastern Spain). The trial site is representative of the average conditions in which the species can be found in the Mediterranean. The mean annual temperature is 13.8 °C, and the mean annual precipitation is 652 mm, of which 19% falls in summer (Jun. to Aug.). Mean annual potential evapotranspiration is 1115 mm. Seeds of *P. halepensis* were collected in 1995 in 56 natural populations covering most of the species' range (Fig. 2; Table S1 [**Supplementary Information**]). In each population, seeds were harvested from 20–30 trees that were spaced at least 100 m apart and planted in a forest nursery in Spain, following standard practices. In 1997, 1-year-old seedlings (16 per population) were planted systematically (2.5 × 2.5 m spacing) at the study site in four replicates following a Latinized row-column design. Four seedlings per population were planted in experimental units consisting of linear plots. A total of 896 seedlings (16 per population) were tested in the trial. Between 2004 and 2017, several field campaigns were performed to characterize the trees for different traits.

Water uptake patterns

The percentage of water taken up from two consecutive soil layers was estimated for each population in Jul. 2010. For this purpose, soil samples at two depths (0–15 cm and 15–40 cm)

were systematically collected, covering all the area of the trial. One healthy and sun-exposed branch per tree was also collected from the top part of the crown and bark-peeled. Branches and soil samples were immediately frozen in dry ice and then stored at −20 °C to prevent evaporation. Water was extracted from the soil and from the xylem by cryogenic vacuum distillation as described in Otieno *et al.* (2006). Prior to water extraction, the branches sampled from trees of the same plot were pooled together. The oxygen and hydrogen isotopic composition ($\delta^{18}O$ and $\delta^{2}H$) of the soil and xylem water was determined by isotope ratio infrared spectroscopy. The relative contributions of water at 0–15 cm (TOP) and 15–40 cm (BOTTOM) to xylem water were estimated based on the isotopic composition of water through Bayesian mixing modelling. A detailed description of the procedure and the original data are reported in Voltas *et al.* (2015).

Chlorophyll content, leaf area and transpiration

Multispectral and thermal images obtained with a UAV (unmanned aerial vehicle) were used to retrieve values of vegetation indices and canopy temperature at plot level, as surrogates of leaf area, chlorophyll content and transpiration. Two flights were performed on 26 Jul. 2016 and 25 May 2017 with a Mikrokopter OktoXL (Moormerland, Germany) flying under remote control at around 100 m of altitude. A multispectral camera (MCA12; Tetracam Inc., Chatsworth, CA, US) and a thermal camera (FLIR Tau2 640; FLIR Systems, Nashua, NH, USA) were mounted, looking down, on the UAV to capture multispectral and thermal images with a resolution of 10 and 25 cm respectively. The raw photographs were combined to produce orthomosaics using a variable number of images with at least 80% overlap. The four orthomosaics (two per flight, one for multispectral and one for thermal images) that resulted from this process were used for the analyses.

The Renormalized Difference Vegetation Index (RDVI) (Roujean and Breon, 1995), the Optimized-Soil Adjusted Vegetation Index (OSAVI) (Rondeaux *et al.*, 1996) and the Transformed Chlorophyll Absorption Ratio Index (TCARI) (Haboudane *et al.*, 2002) were calculated for each pixel of single images corresponding to the experimental units. An average value per plot was obtained afterwards. Also, canopy temperature was measured from the thermal images for each pixel of a single experimental unit and used to calculate the average temperature of the plot. RDVI and OSAVI are vegetation indices based on red and near infrared (NIR) reflectance and have been used as indicators of leaf area (Roberts *et al.*, 2016; Xue and Su, 2017). The TCARI includes also the reflectance at green wavelengths and is negatively related to leaf chlorophyll content, but it is influenced by differences in leaf area as well (Daughtry *et al.*, 2000). A better estimation of chlorophyll content in the needles can be obtained by calculating the ratio between TCARI and OSAVI (Haboudane *et al.*, 2002; Zarco-Tejada *et al.*, 2004). Thus, the TCARI/OSAVI index is negatively related to chlorophyll content. For the sake of simplicity, we multiplied this index by −1 to obtain an index that is positively related to chlorophyll content (hereafter, TCARI/OSAVI*). Canopy temperature is sensitive to changes in leaf area but it is also indicative of transpiration rates related to stomatal conductance (Gonzalez-Dugo *et al.*, 2013).

Prior to calculation of the vegetation indices and canopy temperature, a filter was applied to multispectral and thermal images to remove pixels that mainly contained soil. In the case of the multispectral images, the filter was based on the Normalized Difference Vegetation Index (NDVI; Richardson and Wiegand, 1977). In the case of thermal images, a filter based on an automatic Otsu's classification (Otsu, 1979) was applied. Only those pixels identified as vegetation were used to calculate plot-level values of RDVI, TCARI/OSAVI* and canopy temperature. A detailed account of the methodology and associated results can be found in Santini *et al.* (2019).

Non-structural carbohydrates

In Jun. and Sep. 2010, healthy and sun-exposed branches with an approximate diameter of 1 cm were collected from the top part of the crown for the analysis of non-structural carbohydrates (soluble sugars and starch) in sapwood. The analysis was performed on two different dates to characterize the accumulation of non-structural carbohydrates before (late spring) and after (early autumn) the peak period of drought stress. The branches were frozen in the field in dry ice and then dried in the laboratory. Branches collected from the same plot were bark-peeled, pooled together and finely milled. Soluble sugars were extracted from 50-mg samples with 80% ethanol in a shaking water bath at 60 °C. The concentration of soluble sugars in the supernatant obtained after centrifugation was determined colorimetrically at 490 nm using the phenol–sulphuric method described in Buysse and Merckx (1993). After ethanol extraction, the remaining sample in the undissolved precipitate was digested with an enzyme mixture containing amyloglucosidase to reduce glucose as described in Palacio *et al.* (2007). Starch concentration was determined colorimetrically using the same method as for soluble sugars. Each sample was measured twice to check for repeatability of the protocol.

Growth and reproduction

From 2001, trees were monitored across different growing seasons, and age at first female flowering (first appearing of female strobili) was recorded. In 2004 (at age 7), the number of cones per tree was measured as a surrogate of female reproduction. A detailed description of the sampling protocol and the original data are reported in Climent *et al.* (2008). In 2010 (at age 13), tree height and diameter at breast height (DBH) were registered per tree and were used as measures of tree growth. We assumed similar population rankings in tree growth from age 13 onwards, as previously observed for Aleppo pine (Sbay and Zas, 2018).

Climatic data at geographic origin of the populations

Monthly averages of precipitation and of maximum and minimum temperatures for each geographic origin were obtained for the period 1901–2016 from the CRU TS3.22 dataset (Harris *et al.*, 2014). Mean annual temperature (T_{an}), mean summer (Jun. to Aug.) temperature (T_s), mean maximum temperature of the warmest month (T_{max}), mean minimum temperature of the coldest month (T_{min}), temperature range (T_r, calculated as $T_{max} - T_{min}$), total annual precipitation (P_{an}), summer (Jun. to Aug.) precipitation (P_s) and summer to annual precipitation ratio (P_s/P_{an}) were calculated. Monthly temperatures and precipitation were used to derive the annual potential evapotranspiration (PET_{an}) according to the Hargreaves method (Hargreaves and Samani, 1982). Finally, average vapour pressure deficit (VPD) was calculated from altitude and monthly temperature and precipitation following Ferrio and Voltas (2005).

Statistical analysis

Values of cone count, age at first flowering, DBH, height, TOP, BOTTOM, soluble sugars and starch in spring and autumn, RDVI, TCARI/OSAVI* and canopy temperature were subjected to analysis of variance (ANOVA) for linear mixed-effect models in order to test for population differences. In the case of TOP, BOTTOM, OSAVI, TCARI/OSAVI*, canopy temperature, soluble sugars and starch, which were recorded at the plot level, the ANOVA consisted of population, replicate and column as fixed terms, and column by replicate interaction and row nested to replicate as random terms. In the case of cone count, age at first flowering, DBH and height, which were recorded at the tree level, an extra term accounting for intra-plot variability was included in the ANOVA. For those traits showing significant differences among populations, simple correlations between the populations' least square

means and climatic conditions at origin were calculated. The populations' least square means of the different variables, as derived from the ANOVA, were used to build the Structural Equation Model (SEM) and, hence, disentangle associations among traits. Out of the initial 56 populations evaluated in the trial, 51 were used, for which records of all traits were available.

Model specification

A multivariate matrix consisting of 51 populations and 11 functional and fitness-related traits were used for SEM fitting (Table S2 [**Supplementary Information**]). We considered six latent variables describing key characteristics of Mediterranean forest species: water uptake depth, summer transpiration rate, spring photosynthetic capacity, reserve accumulation, growth and reproduction. Each latent variable was defined based on a set of traits as follows. First, the relative contributions of different water sources according to soil depth (TOP, BOTTOM) were considered as descriptors of the latent variable *Water uptake depth* (Voltas *et al.*, 2015). Second, the latent variable *Summer transpiration* was defined by the RDVI and also by canopy temperature, since the transpiration rate results from the combined effect of transpiring surface (i.e., leaf area) and stomatal conductance (Whitehead, 1998; Eamus *et al.*, 2000). Canopy temperature is negatively related to both leaf area and stomatal conductance, while RDVI is positively correlated with leaf area (Gonzalez-Dugo *et al.*, 2013; Roberts *et al.*, 2016; Xue and Su, 2017). Here we used RDVI and canopy temperature measured in peak summer (Jul. 2016), since canopy temperature measured in spring did not differ among populations (see 'Results' section and Santini *et al.*, 2019). On the other hand, needle chlorophyll content (as indicated by TCARI/OSAVI*) differed only in spring (May 2017), indicating population differentiation early in the growing season (see 'Results' section and Santini *et al.*, 2019). Chlorophyll content is directly related to maximum photosynthetic rate, and TCARI/OSAVI* was therefore used as an indicator for the variable *Spring photosynthetic*

capacity (Gratani *et al.*, 1998; Klein *et al.*, 2013). Fourth, the latent variable *Reserves*, describing the investment in reserves, was defined by the concentration of starch and soluble sugars in branches (Hoch *et al.*, 2003). We only considered the values of soluble sugars and starch recorded in Jun., because there was no population differentiation in the case of soluble sugars and starch measured in Sep. (see 'Results' section). Fifth, we used age at first flowering and cone count as indicators of the latent variable *Reproduction*, since they are related to the precocity in reaching sexual maturity and the investment in reproductive structures (Climent *et al.*, 2008). Finally, the latent variable *Growth* was described by DBH and height measurements (Vizcaíno-Palomar *et al.*, 2016). None of the indicators had fixed path coefficient with the latent variables, with the exception of TCARI/OSAVI*.

Once the model was specified, we tested the relations between latent variables by considering all the possible free covariances among the variables within the groups of (i) source-related variables and (ii) carbon sinks. Moreover, regressions of carbon sinks on each source-related variable were included in the starting model. After fitting the starting model, we tested simplified models in which non-significant relations were removed either alternatively or all at once.

Model fitting and evaluation

Prior to model fitting, the multivariate normal distribution was tested through Mardia's skewness and kurtosis tests implemented in the R package 'MVN' (Korkmaz *et al.*, 2014). The variables cone count and TOP were log-transformed to achieve multivariate normality. Model parameters were estimated through maximum likelihood which maximizes the agreement between observed and predicted variance–covariance matrices. Parameter estimation was performed in the package 'lavaan' (Rosseel, 2012) implemented in R, and the goodness of fit was evaluated through a chi-square test to check for discrepancies between the

model-implied and observed matrices of variance–covariance. Several fit indices were also calculated. The Bentler's comparative fit index (CFI; Bentler, 1990) compares the proposed model with a null model in which the observed variables are uncorrelated. It ranges from 0 to 1, with values > 0.95 indicating a good fit (Hu and Bentler, 1999). The root mean square error of approximation (RMSEA; Steiger, 1990) is a measure of model misspecification, with values higher than 0.06 indicating a non-optimal model (Fan *et al.*, 1999). The standardized root mean square residual (SRMR) is a measure of the difference between the observed and the predicted matrices of correlations, and should not exceed 0.09 (Hu and Bentler, 1999). Finally, the significance of each path in the model was evaluated through a *z*-test testing the null hypothesis that the path has zero value and considering unstandardized path coefficients. A bootstrapped estimate and its associated standard error and confidence interval were also calculated for each path based on 1000 replications.

RESULTS

The population term in the ANOVAs was significant ($P < 0.05$) for all traits, with the exception of soluble sugars and starch concentrations measured in early autumn (Sep. 2010), canopy temperature measured in spring (May 2017) and TCARI/OSAVI* measured in peak summer (Jul. 2016). The population term for starch concentration in late spring (Jun. 2010) was marginally significant ($P = 0.12$), so we opted to include this trait in the SEM. The associations between population means and climatic conditions at the geographic origin of the populations are reported in Table 1. In general, drier conditions at origin were associated with reduced growth, higher cone production and earlier first flowering. A lower level of chlorophyll in spring and reduced transpiration in summer were observed in populations originating from drier areas, as indicated by the associations with TCARI/OSAVI* and canopy temperature Finally, soluble sugar and starch concentrations in spring were positively

correlated with temperature range and negatively associated with minimum annual temperature.

The populations' least square means derived from the ANOVAs can be found in Table S2 [**Supplementary Information**]. This table contains the raw values used as input for SEM. The matrix of correlations between populations' least square means is also reported (Table S3 [**Supplementary Information**]). A chi-square test performed on the starting SEM model was non-significant (χ^2 = 31.70, *df* = 30, *P* = 0.38), indicating good agreement between the model-implied and observed variance–covariance matrices. The goodness-of-fit statistics showed optimal values (CFI = 0.99, RMSEA = 0.03, SRMR = 0.07). On the other hand, a number of regression coefficients between latent variables were not significant. The path coefficients of the starting model are reported in Fig. S1 and Table S4 [**Supplementary Information**]. We simplified this model by excluding all non-significant relations. The variance–covariance matrix implied by the simplified model still showed good agreement with the observed matrix, as indicated by the chi-square test (χ^2 = 35.84, *df* = 35, *P* = 0.43). The goodness-of-fit statistics also indicated a good overall fit (CFI = 1.00, RMSEA = 0.02, SRMR = 0.09). A Likelihood-Ratio test performed on these two models was non-significant, suggesting that the simplified model fitted the data as well as the complex model.

The standardized path coefficients of the simplified model (obtained by re-scaling the selected traits and latent variables to unit variance) are shown in Fig. 3. The non-standardized coefficients, including standard errors, *z*-tests and confidence intervals, are reported in Table 2. In general, the selected traits were good indicators of latent variables at the population level, with absolute standardized path coefficients exceeding 0.80. The only exception was starch concentration, which showed a relatively low coefficient (0.52) with the latent variable *Reserves*. Significant covariation emerged between source latent variables, and also between sink latent variables (Fig. 3; Table 2). *Water uptake depth* and *Summer transpiration* were

negatively related, suggesting that populations having enhanced transpiration in summer used comparatively less water from deeper soil layers. The variable *Spring photosynthetic capacity* was positively related to *Summer transpiration*, indicating a tendency of populations with a higher photosynthetic capacity in spring to show enhanced transpiration in summer. *Spring photosynthetic capacity* was also negatively associated with *Water uptake depth*, although this relation was only marginally significant. Among the sink variables, *Growth* and *Reproduction* and *Growth* and *Reserves* were negatively related. Associations between source and sink variables were relevant in some instances. In particular, *Reproduction* was negatively influenced by *Water uptake depth* and by *Spring photosynthetic capacity*, *Reserves* was positively dependent on *Spring photosynthetic capacity*, and *Growth* was positively influenced by *Summer transpiration*. A marginally (positive) influence of *Spring photosynthetic capacity* on *Growth* was also observed. This relation was kept in the model since its removal penalized goodness-of-fit statistics. The bootstrapped estimates of the path coefficients in the simplified model were similar to those obtained through maximum likelihood (Table S5). While the model explained about 65% of the latent variable *Growth*, it explained poorly the variance of the latent variables *Reproduction* and *Reserves* (Fig. 3), as indicated by low R^2 values.

DISCUSSION

Population differentiation in functional traits

This study combined population records of meaningful morpho-physiological traits of *P. halepensis* derived from different published and unpublished studies performed in a representative common-garden experiment (Climent *et al.*, 2008; Voltas *et al.*, 2008, 2015; Santini et al., 2019). In *P. halepensis*, population differentiation in water uptake patterns, leaf physiology, canopy architecture, growth and reproduction has been thoroughly described in

relation to the relevance of local adaptation, particularly in terms of drought resistance (Climent *et al.*, 2008; Voltas *et al.*, 2008; Klein *et al.*, 2013; Santos-del-Blanco *et al.*, 2013; Santini *et al.*, 2019). In addition to such differences, we also report on the extent of population differentiation in reserve accumulation, a feature not previously investigated for this species. The investment in reserves may represent a demanding carbon sink that can ensure survival in periods of potential carbon starvation, which in the Mediterranean region corresponds to the peak of summer in concord with the highest drought severity (Wiley and Helliker, 2012; García de la Serrana *et al.*, 2015). However, only soluble sugars concentration measured in spring clearly differed among populations. In particular, enhanced concentration was related to continentality (i.e., temperature range) and negatively associated with minimum temperatures at the geographic origin of the populations, indicating an influence of winter harshness on the accumulation of spring reserves (Hoch *et al.*, 2003). This finding suggests that the accumulation of reserves does not play a relevant role in determining adaptation to drought in *P. halepensis*. In this regard, Klein *et al.*, (2014*a*) found a very small variation in non-structural carbohydrates accumulated in branches of individuals of *P. halepensis* exposed to different drought treatments. However, the accumulation of reserves in anatomical compartments other than branches (i.e., roots or main trunk) may be important in providing resources during periods of carbon starvation (Hoch *et al.*, 2003).

Contrasting life history strategies among populations of P. halepensis

SEM described the nature of phenotypic integration adequately, as indicated by goodness-of-fit statistics (Fan *et al.*, 2016). Notably, the three source-related traits (i.e., summer water uptake depth, transpiration in summer, and spring photosynthetic capacity) were highly integrated. Transpiration divergence among populations co-varied with water uptake depth in summer. However, these two traits were negatively related, which indicates that a shallower

water uptake is associated with enhanced transpiration – in terms of higher stomatal conductance and/or larger total leaf area – under moderately water-limited conditions (e.g., those encountered in the common-garden test). Under drought stress, a deeper water uptake is expected to ensure water supply, thus sustaining higher stomatal conductance and a greater transpiring surface (Eggemeyer *et al.*, 2008; Rossatto *et al.*, 2012). *P. halepensis* is known to rely on deep water sources to overcome drought periods (Voltas *et al.*, 2015), but genetic differences in the depth of water uptake are indicative of variation in the investment in roots among populations of this species (Klein *et al.*, 2014b; Voltas *et al.*, 2015). The associations with climatic conditions at origin revealed that populations from (i.e., likely adapted to) drought-prone areas tend to invest more resources in a deeper rooting system and, simultaneously, reduce summer transpiration by physiological (i.e., reduced stomatal conductance) and anatomical (i.e., reduced total leaf area) adaptations (Otieno *et al.*, 2006). These results point to water uptake depth and transpiration as functionally related traits whose population covariation has been likely shaped by such common selective pressure (Armbruster and Schwaegerle, 1996).

Similarly, spring photosynthetic capacity was negatively related to access to deeper water pools and positively associated with summer transpiration. The TCARI/OSAVI* index measured in spring (i.e., indicative of photosynthetic capacity) was negatively associated with potential evapotranspiration and VPD at origin. These findings indicate that populations originating from drought-prone areas are characterized by a reduced photosynthetic capacity in spring along with reduced summer transpiration and a deeper water uptake. In turn, they confirm previous evidence of higher spring chlorophyll content in needles of *P. halepensis* populations originating from Greece, which are among the populations experiencing the wettest growing conditions at origin among those considered in our study (Klein *et al.*, 2013). We hypothesize that *P. halepensis* populations originating from mesic conditions (i.e.,

characterized by lower water uptake depth and higher summer transpiration) have developed more efficient photosynthetic machinery in spring, when photosynthesis may be light- rather than water-limited in Mediterranean ecosystems (Flexas *et al.*, 2014). In this regard, other traits related to photosynthetic capacity (i.e., photosynthetic pigments) have been found to be relatively constant in summer – when water availability is expected to limit photosynthesis across the whole species' range – across populations of *P. halepensis* (Santini *et al.*, 2019).

Significant associations emerged between source-related variables and carbon sinks, although these associations explained a relatively low variance of reproductive and storage patterns. On the other hand, they explained *ca.* 65% of the variation in growth among populations, indicating a good SEM predictive ability of carbon allocation patterns to stem biomass. Indeed, a strong, positive intra-specific association was observed between summer transpiration and growth, confirming that carbon assimilation in Aleppo pine depends more on total needle area and stomatal regulation than on photosynthetic capacity (Voltas *et al.*, 2008). The results of our model indicate that populations originating from conditions enabling higher water use and, therefore, higher transpiration can sustain a higher growth (Fardusi *et al.*, 2016).

Contrarily to growth, reproduction and reserve accumulation were not directly related to summer transpiration, suggesting that differences in summer carbon fixation among populations do not elicit changes in these alternative sinks. On the other hand, a strong and negative covariation between reproduction and growth was noticeable. A high investment in primary and secondary growth for populations of this species is coupled with delayed sexual maturity or low cone yield (Climent *et al.*, 2008; Santos-del-Blanco *et al.*, 2013). This realization emphasizes the evolutionary divergence in growth and reproduction as functionally opposed life history traits in *P. halepensis*, and points to contrasting population strategies in the allocation of resources to such fundamental processes. Indeed, trade-offs in

resource allocation to reproduction or growth are well known in forest species (Obeso, 2002). This differentiation has been linked to particular life history strategies related to growth conditions (Niklas and Enquist, 2002). In general, individuals growing in unstable environments tend to invest more resources in reaching sexual maturity faster at the expense of lower vegetative growth, and vice versa (Santos-del-Blanco *et al.*, 2013).

In Mediterranean forests, fire has been identified as a primary source of ecological instability that acts as evolutionary force in pine species (Pausas, 2015). Differences in the frequency and intensity of forest fires across the range of *P. halepensis* may produce greater investment in reproduction in some populations, in contrast to those exposed to less recurrent fire disturbances (Hernández-Serrano *et al.*, 2014; Martín-Sanz *et al.*, 2016). In this regard, our results indicate that populations showing higher primary and secondary growth, low cone yield and delayed reproduction are those originating from more humid geographic origins, where fire occurrence is expected to be lower (Oliveira *et al.*, 2012). Moreover, a high investment in the rooting system at the population level is obtained at the expense of reduced reproduction, but does not seem to affect growth directly (Voltas *et al.*, 2015). Similarly to growth, a high investment in the root system is typical of trees characterized by a long lifespan, which is evolutionarily relevant in stable environments (Strauss and Ledig, 1985). On the other hand, some populations show reduced root investment coupled with early reproduction, which is indicative of a short lifespan. In these populations, recurrent ecological disturbances such as forest fires may have induced the development of such a strategy (Pausas, 2015). Other fire-related traits such as bark thickness have also been associated with different life strategies in *P. halepensis* (Martín-Sanz *et al.*, submitted) and in other Mediterranean conifers (Resco de Dios *et al*., 2018).

Alongside reproduction, reserves are a third important sink component which may compete with growth in the carbon economy of a tree (Hoch *et al.*, 2003; Körner, 2003).

Broadly speaking, two different models have been proposed to describe the competition between investment in growth and reserves in trees (Wiley and Helliker, 2012): 1) forest species can invest carbon in growth and then use the residual resources to produce reserves (passive accumulation), or 2) they can actively withdraw resources to grow in order to accumulate reserves (active accumulation). Our data reveal intra-specific covariation between growth and reserves, which points to an active model of carbon accumulation in *P. halepensis*.

Spring photosynthetic capacity was the only source-related trait affecting all sink-related traits simultaneously. However, these relations were generally weaker than other source-sink relationships. Specifically, a positive association between photosynthetic capacity and growth emerged, even if much less relevant than the association between summer transpiration and growth. This finding suggests that enhanced spring photosynthetic capacity results in greater allocation of resources to growth in this species (Klein *et al.*, 2013), although a feedback of sink activity on source activity, signalled through the phloem, may also play an important role (Körner, 2014). A positive association between spring photosynthetic capacity and reserve accumulation also emerged from the model, which indicates that photosynthetic products from enhanced spring photosynthesis are (partially) invested in carbon reserves. On the other hand, the negative association between photosynthetic capacity and reproduction might be a consequence of the strong trade-off between reproduction and growth rather than from a direct causal effect. In this regard, the associations between spring photosynthetic capacity and either reproduction or reserves were of the same magnitude, but of opposite sign. Since no significant free co-variance was found between these carbon sinks, this finding indicates that an increase in spring photosynthetic capacity results in an increase of reserves coupled with an equivalent decrease (in terms of carbon resources) of reproduction.

CONCLUSIONS

This work provides strong insights into the array of life history strategies that are found range-wide in *P. halepensis*. The development of complex adaptive syndromes, in which functionally related traits show high phenotypic covariation among populations, has been linked to selective processes (Armbruster and Schwaegerle, 1996). Contrasting selective pressures are likely at the origin of the phenotypic covariation observed among source-related traits, for which a functional integration related to drought adaptation can be postulated. Across the range of the species, fast-growing populations showing high photosynthetic capacity in spring sharply contrast with slow-growing populations having a favourable expression of functional traits related to drought resistance (i.e., deeper rooting system and reduced summer transpiration). These complementary strategies are indicative of evolutionary divergence for the species. On the other hand, the trade-offs that emerged among sink-related traits may be explained in the light of differences in fire regimes, which influence the ecological stability of Mediterranean environments. Slow-growing populations allocate more resources to faster reproduction and to greater accumulation of reserves, which are strategies that have been linked to highly unstable environments characterized by recurrent, intense and widespread forest fires (Niklas and Enquist, 2002; Körner, 2003; Wiley and Helliker, 2012; Pausas, 2015).

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1

FIGURE CAPTIONS

Figure 1. Starting Structural Equation Model. Directional arrows between latent variables indicate regression, while double arrows indicate covariance.

Figure 2. Geographic origin of the 56 *Pinus halepensis* populations (red dots) used in this study and tested in the genetic trial (black dot). Green areas indicate the species range derived from the EUFORGEN distribution map (http://www.euforgen.org/species/pinus-halepensis/).

Figure 3. Final Structural Equation Model. Square nodes denote observed variables, while latent variables are represented by circles. Directional arrows between latent variables indicate regressions, while double arrows indicate covariance. The standardized path coefficients are reported, as well as R^2 values for sink variables significantly explained by source-related variable(s). Significant path coefficients are indicated by * ($P < 0.05$) or ** ($P < 0.01$) according to *z*-tests performed on unstandardized coefficients (Table 2).

TABLES

Table 1

Pearson's correlations between population means of the 11 phenotypic traits considered (i.e. as described in "Material and Methods" section) and climatic conditions at origin. Significant correlations are indicated by * ($P < 0.05$) or ** ($P < 0.01$). Marginally significant correlations ($P < 0.1$) are indicated by $^{+}$.

	T_{an}	T_s	T_{max}	T_{min}	T_r	P_{an}	P_s	P_s/P_{an}	PET_{an}	VPD
BOTTOM	0.03	0.05	0.20	0.02	0.09	−0.07	−0.21	−0.20	0.22	0.19
TOP	0.02	−0.03	−0.22	0.06	−0.17	0.19	0.21	0.16	−0.26^{+}	−0.22
Height	0.12	0.02	−0.35*	0.13	−0.30*	0.05	0.23	0.19	−0.33*	−0.20
DBH	0.09	0.01	−0.26^{+}	0.07	−0.20	−0.11	0.09	0.09	−0.21	−0.11
Soluble sugars	−0.31*	−0.18	0.08	−0.32*	0.33*	0.00	0.09	0.18	0.05	−0.13
Starch	−0.24^{+}	−0.16	0.13	−0.27*	0.31*	−0.21	−0.18	−0.03	0.11	0.02
RDVI	−0.17	−0.08	−0.09	−0.23	0.16	−0.17	0.06	0.14	−0.06	−0.09
TCARI/OSAVI*	−0.37**	−0.33*	−0.26^{+}	−0.33*	0.15	0.16	0.36*	0.39**	−0.36*	−0.40**
Canopy T	0.23	0.28*	0.31*	0.22	−0.03	0.10	−0.23	−0.31*	0.20	0.23
Age first flowering	0.07	−0.09	−0.33	0.13	−0.29*	0.10	0.21	0.19	−0.30*	−0.24
Cone count	0.08	0.24^{+}	0.39	0.05	0.17	0.06	−0.26^{+}	−0.32*	0.32*	0.27*

T_{an} annual temperature; T_s summer temperature (Jun. to Aug.); T_{max} mean maximum temperature of the warmest month; T_{min} mean minimum temperature of the coldest month; T_r range of annual temperature; P_{an} annual precipitation; P_s summer precipitation (Jun. to Aug.); PET_{an} annual potential evapotranspiration; VPD mean vapour pressure deficit.

Table 2

Parameter estimates of the Structural Equation Model. The variables are described in "Material and Methods" section. The estimated non-standardized coefficients are reported. The *z*-statistic corresponds to the estimate divided by its standard error. The *p*-value is calculated by evaluating the *z*-statistic under a standard normal distribution. CI indicates the 95% confidence intervals (lower and upper).

Left variable	Operator	Right variable	Estimate	SE	*z*-value	*p*-value	CI (lower)	CI (upper)
Growth	=~	DBH	1.00	0.00	-	-	1.00	1.00
Growth	=~	Height	1.23	0.09	12.01	0.00	1.05	1.42
Reproduction	=~	Cone count	1.00	0.00	-	-	1.00	1.00
Reproduction	=~	Age first flowering	−0.95	0.18	−5.35	0.00	−1.30	−0.60
Water uptake depth	=~	BOTTOM	1.00	0.00	-	-	1.00	1.00
Water uptake depth	=~	TOP	−0.80	0.10	−8.17	0.00	−0.99	−0.61
Summer transpiration	=~	RDVI	1.00	0.00	-	-	1.00	1.00
Summer transpiration	=~	Canopy T	−3.66	0.51	−7.28	0.00	−4.67	−2.69
Reserves	=~	Soluble sugars	1.00	0.00	-	-	1.00	1.00
Reserves	=~	Starch	0.91	0.37	2.46	0.01	0.18	1.63
Photosynthetic capacity	=~	TCARI/OSAVI*	1.00	0.00	-	-	1.00	1.00
Water uptake depth	~~	Summer transpiration	0.00	0.00	−1.94	0.05	0.00	0.00
Summer transpiration	~~	Photosynthetic capacity	0.00	0.00	2.03	0.04	0.00	0.00
Water uptake depth	~~	Photosynthetic capacity	0.00	0.00	1.82	0.07	0.00	0.00
Reproduction	~	Water uptake depth	−0.14	0.05	−2.88	0.00	−0.24	−0.05
Reproduction	~	Photosynthetic capacity	−0.99	0.45	-2.23	0.03	−1.87	−0.12
Growth	~	Summer transpiration	2.03	0.31	6.57	0.00	1.42	2.64
Growth	~	Photosynthetic capacity	0.51	0.28	1.75	0.08	−0.60	1.07
Reserves	~	Photosynthetic capacity	3.18	1.43	2.22	0.03	0.37	5.99
Growth	~~	Reproduction	0.00	0.00	−2.76	0.01	0.00	0.00
Growth	~~	Reserves	−0.01	0.00	−2.93	0.00	0.00	0.00

=~ latent variable; ~ regressed on; ~~ covariance.

1

2

3

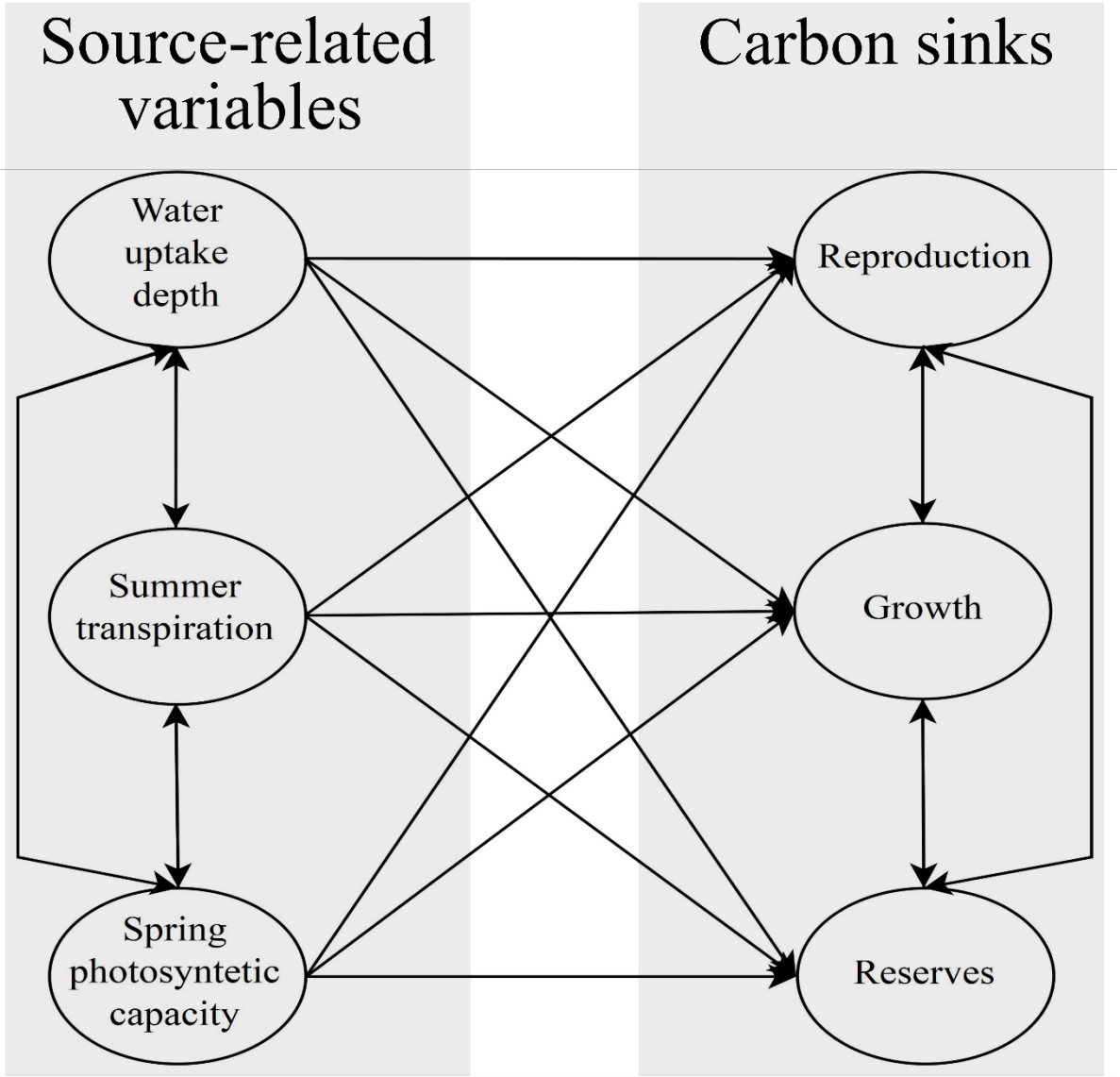
Source-related variables
Carbon sinks
Water uptake depth
Summer transpiration
Spring photosyntetic capacity
Reproduction
Growth
Reserves

Fig 1

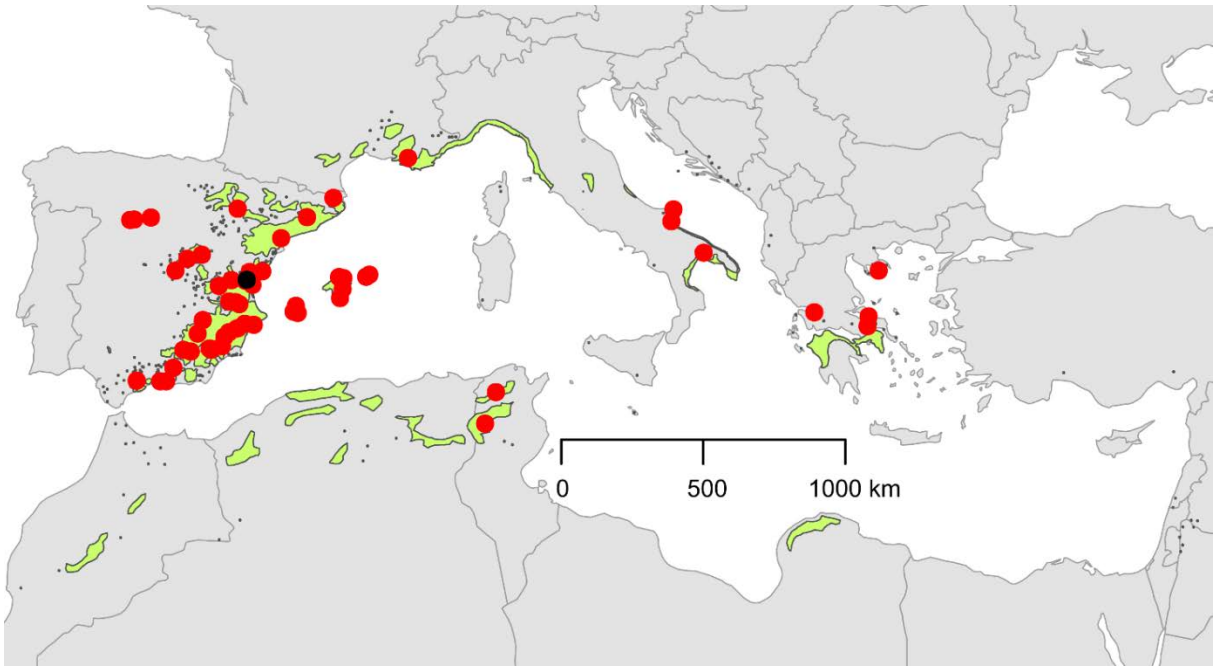

Fig 2

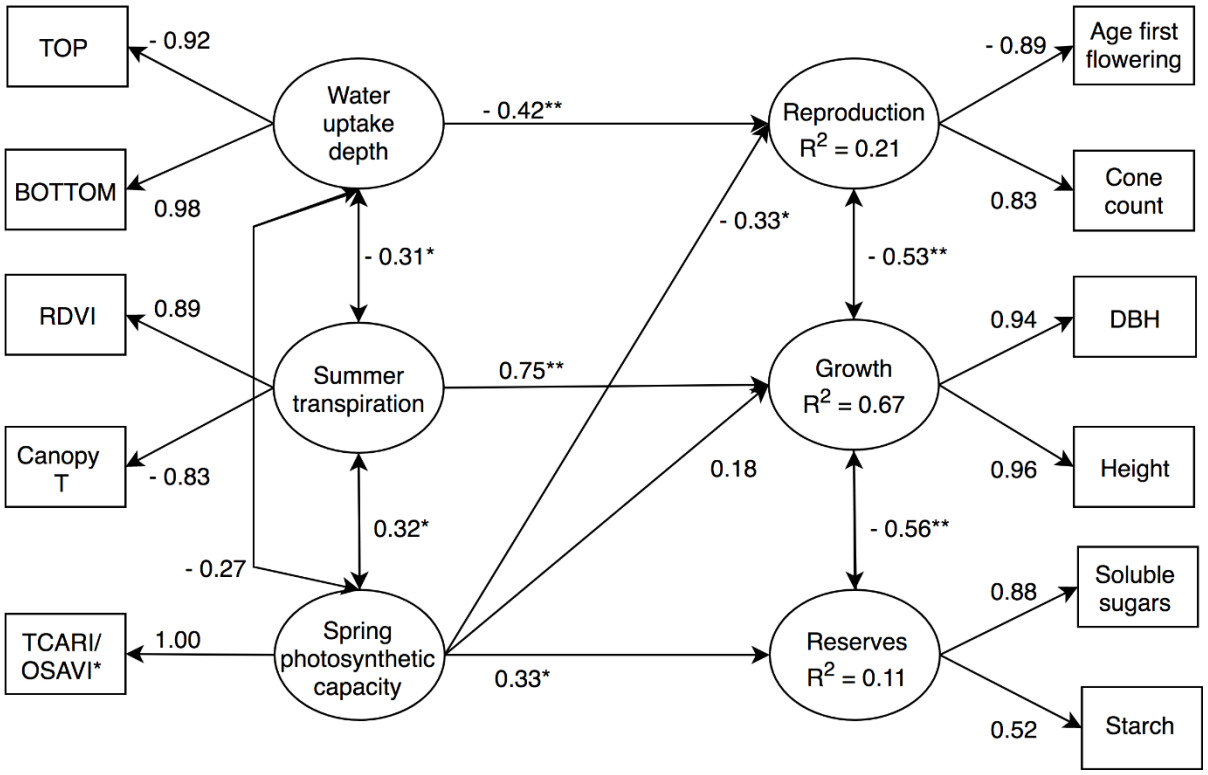
TOP
BOTTOM
RDVI
Canopy T
TCARI/ OSAVI*
Water uptake depth
Summer transpiration
Spring photosynthetic capacity
Reproduction R2 = 0.21
Growth R2 = 0.67
Reserves R2 = 0.11
Age first flowering
Cone count
DBH
Height
Soluble sugars
Starch
- 0.92
0.98
0.89
- 0.83
1.00
- 0.42**
- 0.31*
0.75**
0.32*
- 0.27
- 0.33*
0.18
0.33*
- 0.53**
- 0.56**
- 0.89
0.83
0.94
0.96
0.88
0.52

Fig 3

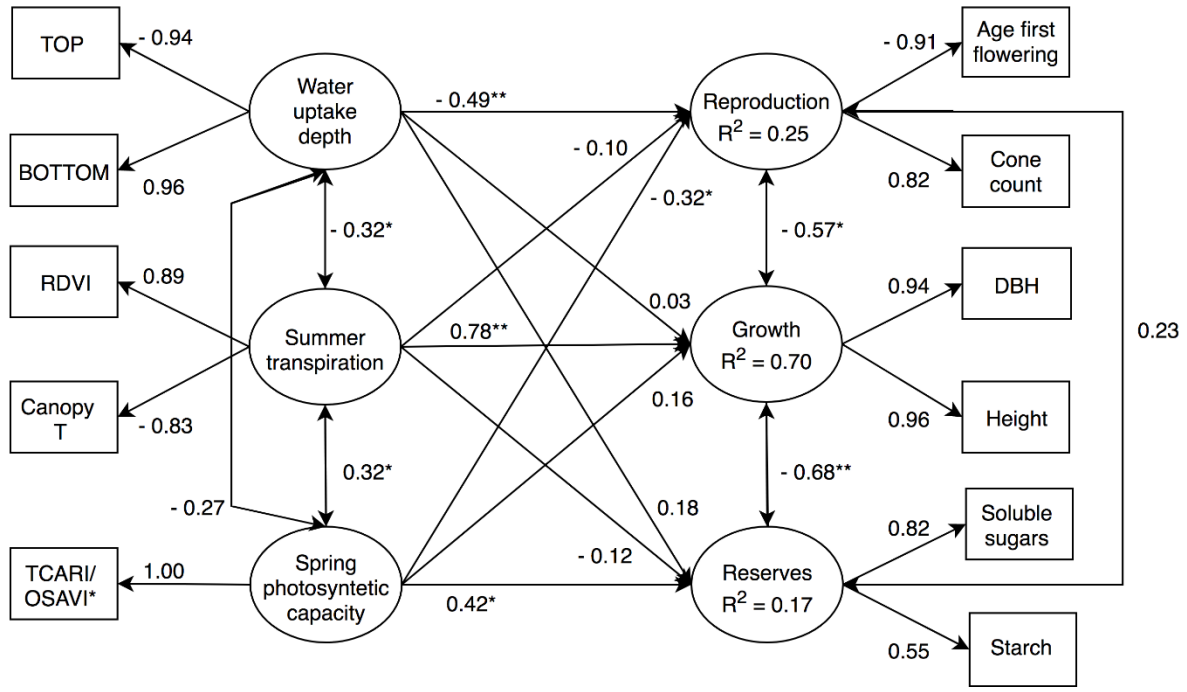
TOP
BOTTOM
RDVI
Canopy T
TCARI/ OSAVI*
Water uptake depth
Summer transpiration
Spring photosyntetic capacity
Reproduction $R^2 = 0.25$
Growth $R^2 = 0.70$
Reserves $R^2 = 0.17$
Age first flowering
Cone count
DBH
Height
Soluble sugars
Starch
- 0.94
0.96
0.89
- 0.83
1.00
- 0.27
- 0.49**
- 0.10
- 0.32*
0.03
0.78**
0.16
0.32*
0.18
- 0.12
0.42*
- 0.91
0.82
- 0.32*
- 0.57*
0.94
0.96
- 0.68**
0.82
0.55
0.23

Fig S1